# Antioxidant and Antihyperlipidemic activity of traditional medicinal plants used in Pakistan to treat hypothyroidism

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## Abstract:

Hypothyroidism is a condition where the thyroid gland doesn't produce enough thyroid hormone. It affects up to 5% of the population, with another 5% going undiagnosed. Studies show that hypothyroidism is linked to increased levels of triglycerides, LDL, and total cholesterol, and lower levels of HDL. This study aimed to evaluate the antioxidant and antihyperlipidemic potential of traditional medicinal herbs used in Pakistan to treat hypothyroidism. Ethanol extracts of *Commiphora wightii*, *Moringa oleifera*, and *Withania somnifera* were tested using total polyphenol, total flavonoid, and DPPH tests. The results showed that *Moringa oleifera* had the highest impact on DPPH radical scavenging activity, while all three extracts and a polyherbal formulation were effective antihyperlipidemic agents. These findings suggest that these traditional medicinal herbs may have therapeutic potential for treating hypothyroidism.

**Keywords:** Antioxidant, Antihyperlipidemic, Hypothyroidism.

## INTRODUCTION:

Hypothyroidism is a medical disorder where the thyroid gland does not generate insufficient thyroid hormone, Hypothyroidism affects up to 5% of the general population, and another 5% are believed to go untreated. Primary hypothyroidism is present in 99% of individuals afflicted[1]. which causes the body's metabolic processes to slow down. [2] Thyroid gland produces hormones that assist control critical bodily processes including inadequate child brain development, infertility, heart rate, body temperature, and metabolism[1, 2].

Hypothyroidism can cause a variety of symptoms; such include sadness, disorientation, indigestion, dry skin, and hair loss, tiredness, weight gain, cold sensitivity, and constipation[3]. Hypothyroidism can occasionally result in myxedema or a goiter. It causes the thyroid gland to expand (a rare, severe form of hypothyroidism)[4]. Other clinical features is hyperlipidemia a condition marked by elevated levels of Low-density lipoprotein cholesterol (LDL), extremely low-density lipoprotein cholesterol, (VLDL) and triglycerides (TG), is now understood to be a sign of hypothyroidism[5].

Diagnosis of hypothyroidism the thyroid profile test is recommended due to evaluate thyroid-stimulating hormone (TSH), which is generated by the pituitary gland and activates the thyroid gland to generate thyroid hormones (T3 & T4), are measured in blood tests used to make the diagnosis[5].
Previous studies have shown that hypothyroidism is linked to raised plasma levels of triglycerides, low-density lipoprotein cholesterol (LDL), and total cholesterol (TC), as well as lower plasma levels of high-density lipoprotein cholesterol (HDL)[5].
The thyroid hormone (TH), on the other hand, has long been known to control the metabolism of plasma total cholesterol however, other research suggested that the thyroid-stimulating hormone (TSH), which is also known to control the metabolism of plasma cholesterol independently of the thyroid hormones, may also do so. This would further advance the development of hyperlipidemia[6].

Antioxidants are compounds that can stop or delay cellular injury brought on by free radicals, which are very reactive molecules created by regular bodily metabolism as well as exposure to external stressors including pollution, radiation, and cigarette smoke[7]. The body's DNA, proteins, and other components can suffer damage from free radicals, which can cause inflammation and a number of disorders[8].
Antioxidants prevent or lessen the harm that free radicals can do by neutralizing them. Natural anti-oxidants found in plants, such as tannins, flavonoids, vitamins C and E, help protect against hypothyroidism-induced cell damage[7]. Several conventional and edible herbs have been used in folk remedies in Pakistan to reduce hypothyroidism symptoms. one of these *Commiphora wightii (Burseraceae)*, also known as mukul or guggul, is a fragrant resin. It is a 4-meter-tall perennial shrub or small tree[9]. Historically, the Guggul has been used to cure conditions including coronary thrombosis, cervical lymphadenitis, diabetes, osteoarthritis, sciatica, hemorrhoids, constipation, skin conditions, inflammation, and urological problems[10]. The steroid guggulsterone, It operates as a farnesoid X receptor antagonist and was originally thought to cause a reduction in cholesterol production in the liver, is one chemical component of the extract[11]. *Withania somnifera L. (Liliaceae*), also known as Indian ginseng, Winter cherry, and Asgandha, is a plant that is found wildly on fields in Asia and can reach a height of about 5 to 6 feet. Its roots, leaves, and many disorders are treated using seeds, including insomnia, nervous breakdown, anxiety, goitre, leucorrhea, boils, pimples, arthritis, lumbago, flatulent colic, and sexual disorders[12]. *Oleifera Moringa (L). (Moringaceae),* often referred to as Sohanjana and Sajina, it is indigenous to Pakistan, Bangladesh, and India, and has been grown throughout the area. It consists of 13 varieties from tropical and subtropical climates, with sizes ranging from little plants to enormous trees.[13] Its leaves are used to cure a wide range of illnesses, including bronchitis, arthritis, anemia, liver damage, hypercholesterolemia, and menstrual irregularities[13 14].

## MATERIAL METHODS

### Medicinal plants material:

The traditional medicines were purchased from a Quetta local market. The Department of Pharmacognosy at Balochistan University in Quetta identified plant components and produced a voucher specimen (Voucher # P037-) that was kept in the herbarium for future use as a reference.

### The extraction processes:

***Commiphora wightii* (CW):** By simply mixing mukul gum powder in 90% ethanol for 7 days, filtration under decreased

pressure, and drying in a vacuum desiccator, *Commiphora wightii* ethanolic extracts were produced. The obtain ratio is 5:1[15]. ***Moringa oleifera* (MO)** The plant's leaves were harvested and dried in the shade in a room. Leaves were crushed and sieved with a 40# sieve after ten days of drying. The Soxhlet device was used to extract Moringa oleifera powder from methanol. The extract was dried at a low temperature in a vacuum evaporator, sterilised using a Whatmann filter no. 42, and reconstituted in saline and condensed under reduced pressure to obtain 5:1 ratio. [16] ***Withania somnifera* (WS)**: root was dried, crushed, and submerged in a variety of solvents (Ethanol). The methanolic filtrates were rotary evaporated after filtration, while the aqueous filtrates were lyophilized with a freeze drier[17].

**Screening of phytochemicals in advance:**

All three crude ethanol extracts underwent preliminary phytochemical screening. Commiphora wightii, Withania somnifera, and Moringa oleifera ethanol extracts were analysed for the presence or absence of phenolics, terpenoids, steroids, glycosides, flavonoids, alkaloids, and flavones and tannins using industry-standard methods[18].

**Experimental samples Preparation:**

Eighteen rabbits were selected in this study, n-6 individuals per group. Group A was the reference group (Control group), receiving a typical food and mineral water, while Groups B and Group C, however, acted as the experimental groups. that were fed a high fatty diet to induce hyperlipidemia for 21 days. After induction Group B receive Polyherbal extract 250 mg diluted in 5 mL/day/oral PHFext and group C served 500 mg diluted in 5mL distal water/day/oral PHFext

**The drug test samples Preparation:**

Each extract mixed equal The test Rabbits were given the polyherbal extract orally after it had been diluted in distilled water with dosage of (250mg and 500mg in 5 mL/kg/day). In the control group, rabbits only ingested distal water (5 mL/kg)[19, 20].

**Experimental animals:**

The Animal Husbandry Sciences Department of Sindh Agricultural University Tandojam's Animal House served as the site of the experimental study. Animal selection criteria were 1.5–2.5 kilograms, 2–3-month-old rabbits. The Animal House Ethics Committee of the Husbandry Sciences Department of Sindh Agricultural University gave its approval to each of the mentioned methods before to the research. For two weeks, the animals were fed regular feed to help them become used to the Animal House environment. The animals were used to the experimental animal station's constant room temperature of 26.0 ℃ and relative humidity range of 40–60%. Both the light and the dark periods of the lighting were maintained[21].

**Developing Hyperlipidemia using standard rabbit chow**

A typical rabbit chow was made with the following ingredients: DL-methionine, calcium phosphate, maize, calcium carbonate, palm kernel meal, starch, molasses, and soybean oil. The pathogenic diet was created by adding 3% saturated fatty acids and 1.3% cholesterol to the regular food, pelleting it, and drying it overnight at 45–50 C.[22, 23]. For the purpose of developing hyperlipidemia, regular rabbit chow was provided for 21 days. A blood sample was taken from the central ear artery after three weeks, and the serum was obtained. A lipid profile (triglycerides, total cholesterol, VLDL, LDL, and HDL) test was performed to evaluate lipid variation in rabbits with induced hyperlipidemia[24].

**Biochemical assays**

The biochemical analysis was done to determine the levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) in the serum. Every assay was carried out on a diagnostic kit-equipped Automated Randox Daytona

analyzer from Randox Crumlin in the UK (Randox Laboratories Limited, Crumlin, United Kingdom)[25].

**Anti Oxidant activity:**

Testing substances of various concentrations (250, 500, and 1000 g/ml) in methanol were dissolved in 2 ml of freshly generated 0.1 mM DPPH radical solution in methanol. After giving the mixture a good shake, it was left to remain at room temperature in the dark for 30 minutes. The same conditions were maintained for the DPPH blank and standard compounds. Using a UV/visible spectrophotometer, the absorbance at 517 nm was measured in triplicate. The following equation was used to compute the percentage of DPPH scavenging using the absorbance values of the test, standard, and blank[26].

DPPH scavenging effect (%) = Ao - A1/Ao × 100

Where: Ao = absorbance of blank, A1= absorbance of test

**Analytical Statistics:**

Dunnett's test and to analyse the data, one-way analysis of variance (ANOVA) was used test, and values with p0.05 were considered significantly different.

**Results:**

Table 1 is showing variation in control group and B group. This table show that the total cholesterol level was significantly high ($p$ > 0.05) in Group-B hyperlipidemia induce compared to the control group, and the presence of cholesterol a dramatic drop in level ($p$ > 0.001) in Group-B hyperlipidemia treatment (PHFext) and This table show that the amount of triglycerides significantly raised ($p$ > 0.05) in Group-B hyperlipidemic induce when in comparison to Group-A Basic control, and Triglycerides there was a dramatic drop in level ($p$ > 0.001) in Group-A treatment (PHFext), This table show that the HDL there was a dramatic drop in level ($p$ > 0.05) in group-B hyperlipidemic induce, when in comparison to the group-A basic control, and HDL was markedly increased ($p$ > 0.05) in Group-B treatment (PHFext), and The LDL level was markedly raised ($p$ > 0.05) in Group-B hyperlipidemic induce when in comparison to the Group-A (control), and level of LDL was very markedly dropped ($p$ > 0.001) in Group-B treatment (PHFext) the total lipid level was very markedly increased ($p$>0.05) in Group-B hyperlipidemic induce when in comparison to the Group-A (control), and the level of total lipid was very markedly decreased ($p$ > 0.001) in Group-B treated with (PHFext).

Table 2 showing variation in Group-A (control) and Group-C. This table show that the total cholesterol level was markedly increased ($p$ > 0.05) in Group-C hyperlipidemia induce when in comparison to the Group-A (control), and level of cholesterol dramatically dropped ($p$ > 0.001) in Group-C hyperlipidemia treatment (PHFext) and this table show that the Triglycerides level was significantly raised ($p$ > 0.05) in Group-B hyperlipidemia induce when in comparison to the Group Basic control, and level of Triglycerides was dramatically dropped ($p$ > 0.001) in Group-C treated with (PHFext), This table also show that The amount of HDL was markedly reduced. ($p$ > 0.05) in Group-B hyperlipidemia induce when in comparison to control group, and level of HDL was significantly raised ($p$ > 0.05) in group-C treated with (PHFext), and the level of LDL was significantly raised ($p$ > 0.05) in group-B hyperlipidemia induce when in comparison to the group Basic control, and level LDL was very markedly dropped ($p$ > 0.001) in group-C treated with (PHFext) and the total lipid level was very significantly raised ($p$ > 0.05) in group-B hyperlipidemia

induce when in comparison to group Basic control, and level of total lipid was markedly dropped ($p$ > 0.001) in group-C treated (PHFext).

**Phytochemical results:**

Table 3 showed the preliminary phytochemical screening results shows that Alkaloid and Flavonoids are present in all three herbs, while Tannins, Saponins, & Phenolics are present in *Withania somnifera & Moringa oleifera* and others side *Steroids, & Terpenoids are present in Commiphora wightii & Withania somnifera* and only Glycosides are present in only *Commiphora wightii*.

**Anti-Oxidant results:**

Table # 4 and figure (1, 2, 3 & 4), shows that the herbal mixture (Polyherbal formulation) proved an anti-oxidant effect with an $IC_{50}$ of 821.2 ppm that was comparable to that of the *Withania somnifera* (WS), which had $IC_{50}$s of 830.09 and 635.93 ppm. However, they proved more anti-oxidant effects (almost 2.5 times as much) when compared to the Commiphora wightii (CW), which had an $IC_{50}$ of 1824. Only the Moringa oleifera (MO), which has an $IC_{50}$ of 168.4 ppm, had good anti-oxidant benefits.

**Discussion:**

Hyperlipidemia is well recognised as a substantial risk factor for the onset and development of atherosclerosis and cardiovascular disease[27]. Hyperlipidemia because they can lead to Either a thrombotic lipoprotein profile linked to a higher chance of developing coronary heart disease (CHD) or acute hyperlipidemia associated with the chylomicronemia syndrome and an elevated chance of acute pancreatitis and increased blood cholesterol and triglyceride levels will almost certainly result in coronary atherosclerosis. As a result, in hyperlipidemia therapy, sustaining cellular cholesterol homeostasis is a crucial factor. When serum lipid concentrations are decreased by hypolipidemic medications, the clinical symptoms of atherosclerosis may be minimized. Therefore, the current situation necessitates research into effective and safe hyperlipidemic medications derived from natural resources [27, 28]. Herbal medicine research has become more important in the treatment of many metabolic illnesses in recent years due to its efficacy and safety. This study's objective is to investigate the possibility for hypolipidemic effects of a polyherbal formulation.

Some researcher reports that *Commiphora wightii* (Guggle) administration has been linked in Ayurvedic scriptures to diarrhoea, menstrual irregularities, skin rashes, headaches, moderate biliousness, and liver damage at extreme dosages[10]. Some other researcher literature show that *Commiphora wightii* (Guggle) have properties with compound preparation effective for Sciatica, hemiplegia, gout, rheumatic disorders, facial paralysis, wound cleaning and healing due to its antimicrobial effect [29, 30]. The sapogenins, pectin component of fenugreek, flavonoids, amino acid, and presence of 4-hydroxyisoleucine could all contribute to the fenugreek extract lipid-lowering impact. [31]. Due to the availability of a wide variety of attempts, the hypolipidemic activity of gum guggul has been validated, leading to the identification of substances such as the potent hypolipidemic compounds gum guggul's isomers E- and Z-guggulsterone[32].

Researcher show that *Moringa oleifera* in indigenous medical system uses different elements of this plant, including the juvenile pods, flowers, fruit, roots, bark, and leaves to treat a variety of illnesses. These parts also have diuretic, anticancer, potential antioxidant, antipyretic, antiepileptic, CNS depressive, anti-inflammatory, antifungal, antimicrobial, antispasmodic, Antifertility,

and antihyperlipidemic Antihepatotoxic, Antiulcer, antidiabetic and anti-epileptic properties [33] [34]. Other researcher mentions that *M. oleifera's* hypolipidemic impact might be brought on by its chemical composition, which includes cardiac glycosides, flavonoids, alkaloids, and saponin. Animal studies have indicated that certain substances can lower blood cholesterol levels[35]. These substances are known to have antioxidant capabilities, which help to inhibit free radical formation, that the risk of cardiovascular disease can be decreased in persons by lowering LDL cholesterol levels and raising total HDL cholesterol[36].

Some researcher mentions in their research articles that *Withania somnifera* have Anti-asthmatic, Rheumatoid arthritis discomfort, anti-inflammatory, immunological booster, Anticancer, Antipyretic, as well as polycythemia and hair melanin properties and *Withania somnifera* is good for sleeplessness but does not act as a sedative. It also stabilizes blood sugar and decreases cholesterol.[37] [38]. The therapeutic benefits of Polyherbal formulation could be attributed to the mixture of alkaloids, withanolides, flavonoids, and catechins identified in W. somnifera[39].

Another research information available in the literature that *Trigonella foenum-graecum* have properties of Gastric stimulant, carminative, antidiabetic, galactagogue, antioxidant, hepatoprotective, antilipidemic, antibacterial, antifungal, antiulcer, antilithigenic, anticarcinogenic, anti-bronchitis, antipyretic, sore throat, anti-inflammatory, anti-dermatitis and irritation, and anti-diabetic [40]. Additionally, *Trigonella foenum-graecum* is utilised for bakery goods, candies, gelatin puddings frozen dairy goods, relish, Pickles, meat products, and sauces[41] [42].

The antihyperlipidemic properties in polyherbal formulation are confirmed in this investigation. When PHFext was given to rabbits on a diet heavy in fat to induce hyperlipidemia (Experimental-Groups), Compared to the control group, serum levels of LDL cholesterol, triglycerides, as well as total cholesterol all significantly lower. Additionally it was observed that the amounts of HDL cholesterol had dramatically increased in both Groups B and Group C.

At the end of this study, both groups' lipid profile values were significantly lower, but Group-C got the 500mg PHFext for 45 days, whereas HDL ($P > 0.001$) was substantial raised in Group-C when compared to Group-B served dosage 250mg PHFext for 45 days.

Oxygen has two opposing characteristics. While oxygen is necessary for life, it also damages cells physiologically through an oxidative reaction [43]. An imbalance between antioxidant and pro-oxidant responses inside living organisms is the primary factor to oxidative and nitrosative stressThere is an overabundance of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and there is a lack of both enzymatic and non-enzymatic antioxidants[44]. As antioxidants, *Commiphora wightii* (CW), *Moringa oleifera* (MO), and *Withania somnifera* (WS) herbal products and their combination (Polyherbal formulation) were also studied. This research used a non-enzymatic free radical biological generating approach to examine three herbal components and their combination in relation to their anti-oxidant potential. In this instance, DPPH was frequently utilised to produce non-enzymatic free radicals[45].

The current study showed that *Withania somnifera* (WS) exhibited $IC_{50}$ values of 830.09 and 635.93 ppm, but the herbal combination (Polyherbal formulation) had an anti-oxidant activity with an $IC_{50}$ of 821.2 ppm. Nevertheless, as compared to the *Commiphora wightii* (CW), which had an

IC50 of 1824, they demonstrated higher anti-oxidant benefits (nearly 2.5 times as much). Only *Moringa oleifera* (MO), with an $IC_{50}$ of 168.4 ppm, provided significant antioxidant advantages.

Herbs' phytochemical component is responsible for their antioxidant capabilities. According to recent research, several phytochemical components found in plants are more potent antioxidants and may thus considerably contribute to the protective benefits[46].

## Conclusion:

It's that the combination of the most active extracts from all four plants can help to lower hyperlipidemia levels. The most effective dosage is 500mg per kg/oral/day. Furthermore, more research is needed to isolate, purify, and characterize active component(s) from most active extracts that could be used as an independent and/or supplementary treatment for Hyperlipidemia.

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| **Table 1: Baseline lipid profile parameters before and after experimental supplementation in rabbits, group A and group B (PHFext=250mg)** | | | |
|---|---|---|---|
| Each group (n=6) | | | |
| **Lipid profile** | Group A (Mean ± SD) | Group B (induced) | Group B ( PHFe) |
| Total Cholesterol | 69.1666±11.8560** | 90.8333±6.7057 | 32.1667±2.4013** |
| Triglycerides | 124.5±8.4557** | 271.3333±56.8881 | 96.6667±14.1374* |
| HDL | 20.5±3.2710** | 23.00±5.2153 | 28.00±2.6076* |
| LDL | 40.8333±6.0470** | 72.5±5.0099 | 52.5±4.2399 |
| Lipid | 251.1666±50.0536** | 705.5±90.9685 | 193.0±61.3123 |

Note: Water ad libitum on a regular diet; values are stated as Mean ± SD, the one-way ANOVA and Dunnet test were used to analyse the results. ** $p < 0.005$ highly significant; * $p < 0.05$ just significant.

| **Table 2: Lipid profile before and after experimental supplementation in rabbits, Control group A , group B, and group C (PHFext=500mg).** | | | |
|---|---|---|---|
| Each group (n=6) | | | |
| **Lipid profile** | Group A (Mean ± SD) | Group B (Mean ± SD) | Group C (Mean ± SD) |
| Cholesterol | 69.1666±11.8560 | 89.6667±5.5015 | 40.0±5.513** |
| Triglycerides | 124.5±8.4557 | 324.8333±77.9908 | 38.50±7.476** |
| HDL | 20.5±3.2710 | 22.00±3.2249 | 30.33±3.559** |
| LDL | 40.8333±6.0470 | 70.1465±4.8793 | 30.50±4.722* |
| Lipid | 251.1666±50.0536 | 718.00±65.6658 | 223.667±72.7754 |

Note: Water ad libitum on a regular diet; values are stated as Mean ± SD, the one-way ANOVA and Dunnet test were used to analyse the results. ** $p < 0.005$ highly significant; * $p < 0.05$ just significant.

**Table 3: Presence of key (Phytochemical) components in ethanolic extract of herbs (*Commiphora wightii, Withania somnifera & Moringa oleifera*).**

| | *Commiphora wightii* | *Withania somnifera* | *Moringa oleifera* |
|---|---|---|---|
| Alkaloids | +ev | +ev | +ev |
| Glycosides | +ev | -ev | -ev |
| Flavonoids | +ev | +ev | +ev |
| Tannins | -ev | +ev | +ev |
| Steroids | +ev | +ev | -ev |
| Saponins | -ev | +ev | +ev |
| Phenolics | -ev | +ev | +ev |
| Terpenoids | +ev | +ev | -ev |

**Table 4: Anti-oxidant effects of the herbal mixture (Polyherbal formulation) and individual herbs extracts IC50+ SEMa(µg/ml).**

| **S.No.** | Compounds | $IC_{50}$+ SEMa(µg/ml) |
|---|---|---|
| **1** | Commiphora wightii (CW) | 1824.15 |
| **2** | Moringa oleifera (MO) | 168.4 |
| **3** | Withania somnifera (WS), | 830.06 |
| **5** | Polyherbal formulation (PHFx) | 821.2 |

**Figure 1: Anti-oxidant properties of polyherbal formulation's and individual herb extracts' ($IC_{50}$+ SEMa(g/ml)).**

Graph # 1 (Anti-oxidant effects of Commiphora wightii)

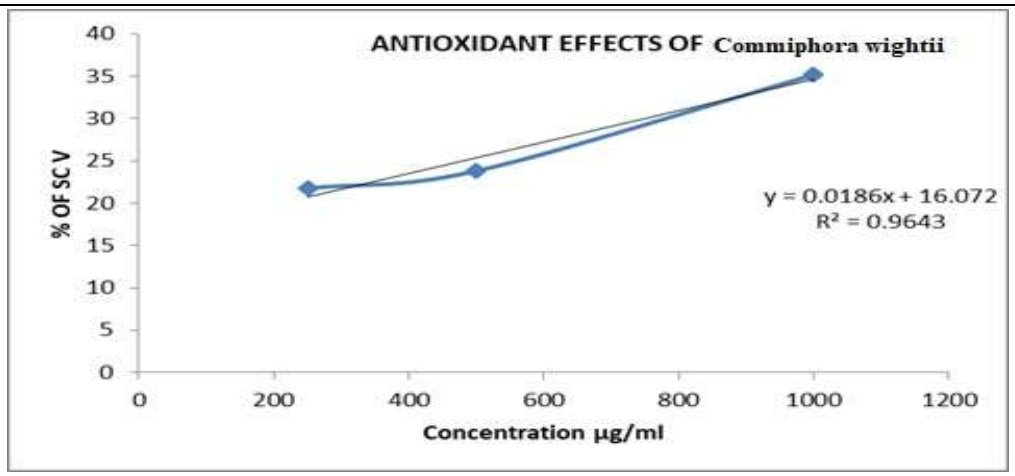


**Figure 2: Anti-oxidant effects of *Moringa oleifera***

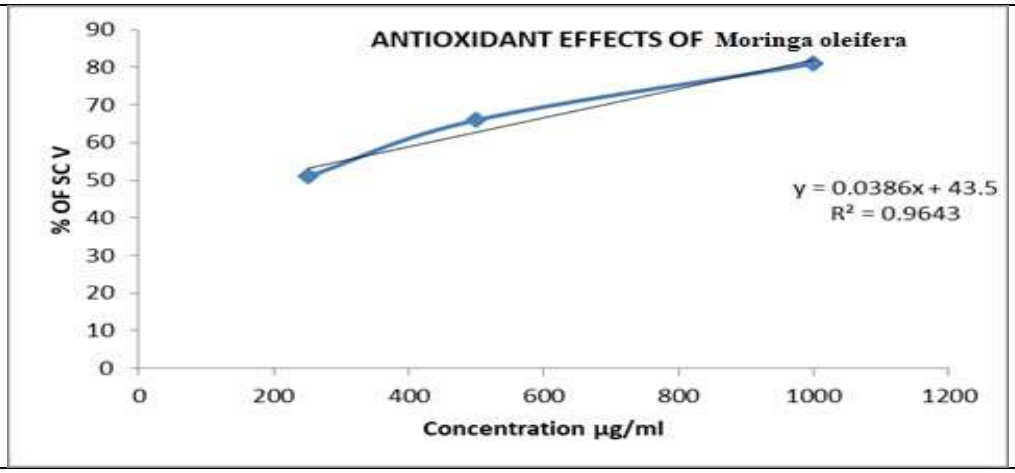


**Figure 3: Anti-oxidant effects of *Withania somnifera***

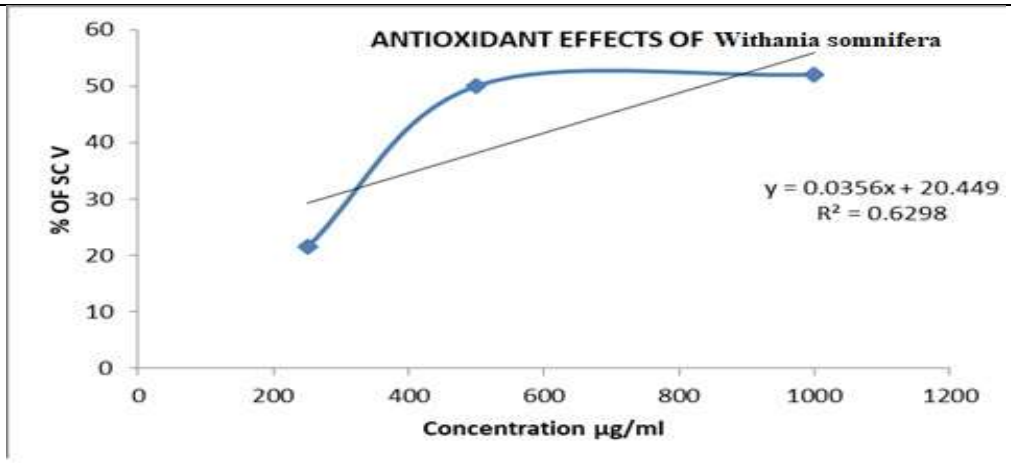

**Figure 4: Anti-oxidant effects of Polyherbal formulation "PHFx"**